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Oligomerizing ability of newly made human Hsp60 with its mitochondrial import signal

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It is currently accepted that the human Hsp60 resides and works not only in the mitochondria, the canonical residence, but also outside it. It is also known that Hsp60 although coded by a nuclear gene is synthesized in the cytosol and includes an N-terminal mitochondrial import signal (MIS), which directs the polypeptide toward the inside of the organelle where the MIS is removed. Therefore, there are at least two functional types of Hsp60, with and without MIS, the former in the cytosol the latter inside the mitochondria. A key question is: how do these two forms of Hsp60 differ beyond the fact that while one has MIS the other lacks it? How presence or absence of MIS affects the ability of Hsp60 to form oligomers, which are considered important for chaperoning peptides and assist them to reach a native state? We report here our initial observations on this issue. Typically, in the mitochondria, Hsp60 forms ring-shaped heptamers, two of which associate to build a barrel-shaped tetradecamer, the functional chaperoning complex. It is not known if the cytosolic Hsp60 with its MIS, also forms hepta- and tetradecamers. A clarification of this issue will most likely shed light on the physiological functions of extramitochondrial Hsp60, and also on its pathogenic role in Hsp60 chaperonopathies. Consequently, we compared recombinant Hsp60 bearing the MIS with the prokaryotic ortholog GroEL, which under normal conditions forms functional double-ring tetradecamers. Characteristic hydrodynamic sizes of the oligomeric complex for both systems were investigated by small angle X-ray (SAXS) and static and dynamic light scattering (SLS and DLS) in solution under similar physicochemical conditions. High Performance Liquid Chromatography (HPLC) and blue native polyacrylamide-gel electrophoresis were used to further clarify the equilibrium between the different oligomeric species of the two proteins over a wide range of concentrations. Hsp60 with MIS formed hepta- and tetradecamers similarly to GroEL. Oligomerization was dependent on concentration for GroEL and Hsp60, but for the latter, formation of larger oligomers, e.g., tetradecamers, required higher concentrations than the former.